

The PITSLRE/CDK11^{P58} protein kinase promotes centrosome maturation and bipolar spindle formation

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The *CDK11* (cyclin-dependent kinase 11) gene has an internal ribosome entry site (IRES), allowing the expression of two protein kinases. The longer 110-kDa isoform is expressed at constant levels during the cell cycle and the shorter 58-kDa isoform is expressed only during G2 and M phases. By means of RNA interference (RNAi), we show that the *CDK11* gene is required for mitotic spindle formation. *CDK11* RNAi leads to mitotic checkpoint activation. Mitotic cells are arrested with short or monopolar spindles. γ -Tubulin as well as Plk1 and Aurora A protein kinase levels are greatly reduced at centrosomes, resulting in microtubule nucleation defects. We show that the mitotic CDK11^{P58} isoform, but not the CDK11^{P110} isoform, associates with mitotic centrosomes and rescues the phenotypes resulting from *CDK11* RNAi. This work demonstrates for the first time the role of CDK11^{P58} in centrosome maturation and bipolar spindle morphogenesis.

Keywords: mitosis; polo-like kinase; γ -tubulin; Aurora A

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INTRODUCTION

The PITSLRE protein kinases belong to a subfamily of p34^{cdc2}-related protein kinases. Ten years ago, PITSLRE protein kinases were renamed CDK11 (cyclin-dependent kinase 11) because of their possible interaction with the regulatory subunits, cyclins L (Xiang *et al*, 1994). Two proteins, CDK11^{P110} and CDK11^{P58}, are translated from a single transcript (Cornelis *et al*, 2000). The CDK11^{P110} protein kinase is present at constant levels during the cell cycle. The CDK11^{P110} isoform is involved in pre-RNA splicing and possibly in the regulation of RNA transcription (Trembley *et al*, 2002). In contrast, the smaller CDK11^{P58} isoform is cell-cycle regulated, and its synthesis occurs through an internal

ribosome entry site (IRES), which is used only during the G2/M transition (Cornelis *et al*, 2000). Thus, CDK11^{P58} protein kinase levels become maximal shortly before entry into mitosis.

In this study, we have characterized the behaviour of human tissue culture cells following CDK11 protein knockdown using RNA interference (RNAi). We show that CDK11^{P58} is a kinase that is, unlike the longer CDK11^{P110} isoform, active during mitosis when it associates with centrosomes, and has a crucial role in maturation of this organelle and bipolar spindle formation.

RESULTS

CDK11 localizes to nuclear punctae and centrosomes

We began our investigation into *CDK11* function by raising a polyclonal antibody that recognizes CDK11^{P110} and CDK11^{P58}. Protein samples from control (Fig 1A, lanes marked –) or RNAi-treated cells (lanes marked +) were analysed by western blot. Whereas CDK11 proteins were specifically depleted, γ -tubulin levels remained unaffected. These observations confirm the specificity of our CDK11 antibody and indicate that RNAi allows efficient CDK11 ‘knockdown’ in HeLa cells.

We next examined the subcellular localization of CDK11 throughout the cell cycle (Fig 1B). The CDK11 antibody decorated punctae in interphase and early prophase nuclei and was also seen diffusely throughout the cytoplasm (Loyer *et al*, 1998) and faintly at the centrosomes (arrows). Progression into prophase was marked by an increase in the centrosomal signal. Centrosome staining increased further and seemed to be maximal at prometaphase and metaphase. During telophase and cytokinesis, the kinase re-accumulated in the karyomeres and a low level of centrosome staining remained. Thus, either one or both of the CDK11 protein kinase isoforms preferentially concentrate on mitotic centrosomes.

CDK11 is required for centrosome maturation

The recruitment of CDK11 to mitotic centrosomes raised the possibility that *CDK11* might have a role in cell division. To test this hypothesis, we studied *CDK11*’s function using RNAi. Three days after short interfering RNA (siRNA) transfection, CDK11^{P110} and CDK11^{P58} protein levels could be reduced by up to 90% (Fig 1A) on western blots, and staining was abolished in fixed cells (not shown). Control and RNAi-treated cells were fixed and

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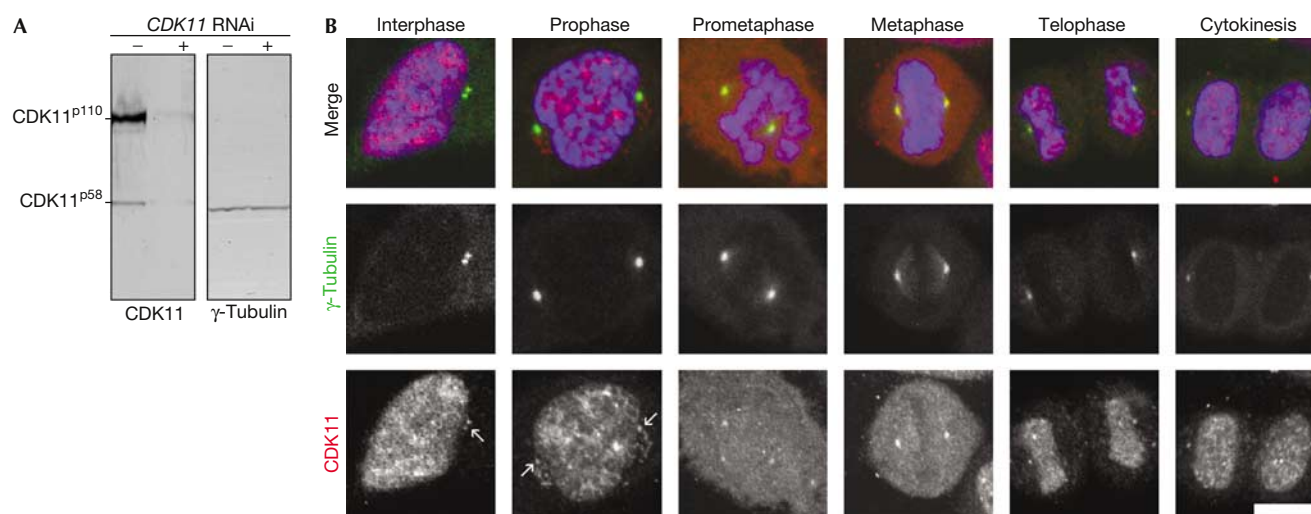


Fig 1 | Cyclin-dependent kinase 11 antibody and localization. (A) Western blot showing cyclin-dependent kinase 11 (CDK11; left) protein levels in control (–) and *CDK11* RNA interference (RNAi)-treated cells (+). Treatment depletes CDK11 but not the γ -tubulin loading control (right). (B) Immunolocalization of CDK11 protein kinases in HeLa cells throughout the cell cycle. In interphase and early prophase, CDK11 (red and lower panels) is sequestered into nuclei, in which it forms distinct speckles/punctae on the DNA (blue). Colocalization with γ -tubulin (green and middle panels) shows that CDK11 associates with centrosomes throughout the cell cycle. Centrosomal labelling of CDK11 increases as the bipolar spindle forms and the chromosomes congress. This signal diminishes as cells transit into anaphase. During telophase and cytokinesis, CDK11 again becomes concentrated within the reforming nuclei. Scale bar, 10 μ m.

stained for microtubules, DNA and centrosomes (Fig 2). The proportion of cells at each of the mitotic stages (Table 1) and the consequences of *CDK11* depletion on the spindle were examined (see below).

At 48 h after *CDK11* RNAi, the number of multinucleated interphase cells increased from 3.7% to 7.9%. A greater increase was observed 72 h after RNAi, and the number of multinucleated cells was elevated by almost five times to that seen in controls. The mitotic index increased by factors of 2 and 3.5 at 48 and 72 h after *CDK11* RNAi treatment, respectively. Depleting *CDK11* raised the number of abnormal mitoses to about 61% of the total dividing cells examined (Table 1).

At 48 h after *CDK11* RNAi, about 23% of the mitotic cells consisted of normal bipolar spindles to which one or more chromosomes seemed to linger at the spindle poles, giving an exaggerated prometaphase-like state (Fig 2B). At 72 h after treatment, in about 61% of the mitotic cells examined, the spindles were abnormally short or monopolar, with hypercondensed chromosomes (Fig 2B). The kinetochores in *CDK11* RNAi-treated cells stained positive with the BubR1 checkpoint protein, suggesting improper attachment. Consistent with this, we detected another BubR1-positive band on western blots following *CDK11* RNAi, which is a signature of checkpoint activation (Chan *et al*, 1999; supplementary Fig S1A,B online). These observations indicate that depletion of *CDK11* affects the spindle shape, leading to checkpoint activation and mitotic arrest.

We also noticed a reduction in γ -tubulin levels at the centrosomes in *CDK11* knockdown cells relative to similarly staged prometaphase control cells (compare Fig 2A and B, insets), and therefore examined the recruitment of γ -tubulin in fixed cells. During prophase in control cells, the γ -tubulin signal at the centrosomes was 4–5 times higher than that in G2 (Fig 2C), and

recruitment continued to increase during the first portion of mitosis, such that by late prometaphase the levels were about seven times higher than those seen in G2 (Fig 2C). Although total cellular levels of γ -tubulin were similar between control and *CDK11* RNAi cells (Fig 1A), the γ -tubulin signal intensity at the centrosome did not increase beyond that seen in control G2 cells, irrespective of mitotic stage or spindle morphology (Fig 2C). These data strongly suggest that γ -tubulin is not properly recruited to the centrosomes in dividing *CDK11*-depleted cells.

Microtubule nucleation is facilitated by CDK11

To determine whether the short/monopolar spindles observed after *CDK11* RNAi resulted from a failure in centrosome separation or subsequent spindle collapse, we used time-lapse imaging of HeLa cells expressing green fluorescent protein (GFP)-tagged α -tubulin (Fig 3A). In control cells, the time between prometaphase onset and anaphase entry was 90 ± 12 min ($n = 15$; Fig 3A; supplementary Movie 1 online). During this period, the previously separated centrosomes and associated asters formed a bipolar spindle that reached a steady-state metaphase length in about 20 min. The centrosomes were also separated following mitotic entry at prophase in cells 72 h after *CDK11* RNAi treatment (Fig 3A, panel 0 min; supplementary Movie 2 online). However, they did not undergo further separation and, in some instances, they seemed to become more closely opposed during the 2 h filming period ($n = 17$), leading to the short spindles observed in our fixed cell studies. Therefore, *CDK11* mediates bipolar spindle morphology by promoting centrosome separation.

As prometaphase centrosome separation is a microtubule-mediated event and *CDK11* RNAi hinders centrosomal recruitment of γ -tubulin, we investigated whether the microtubule nucleating potential of this organelle was compromised. We

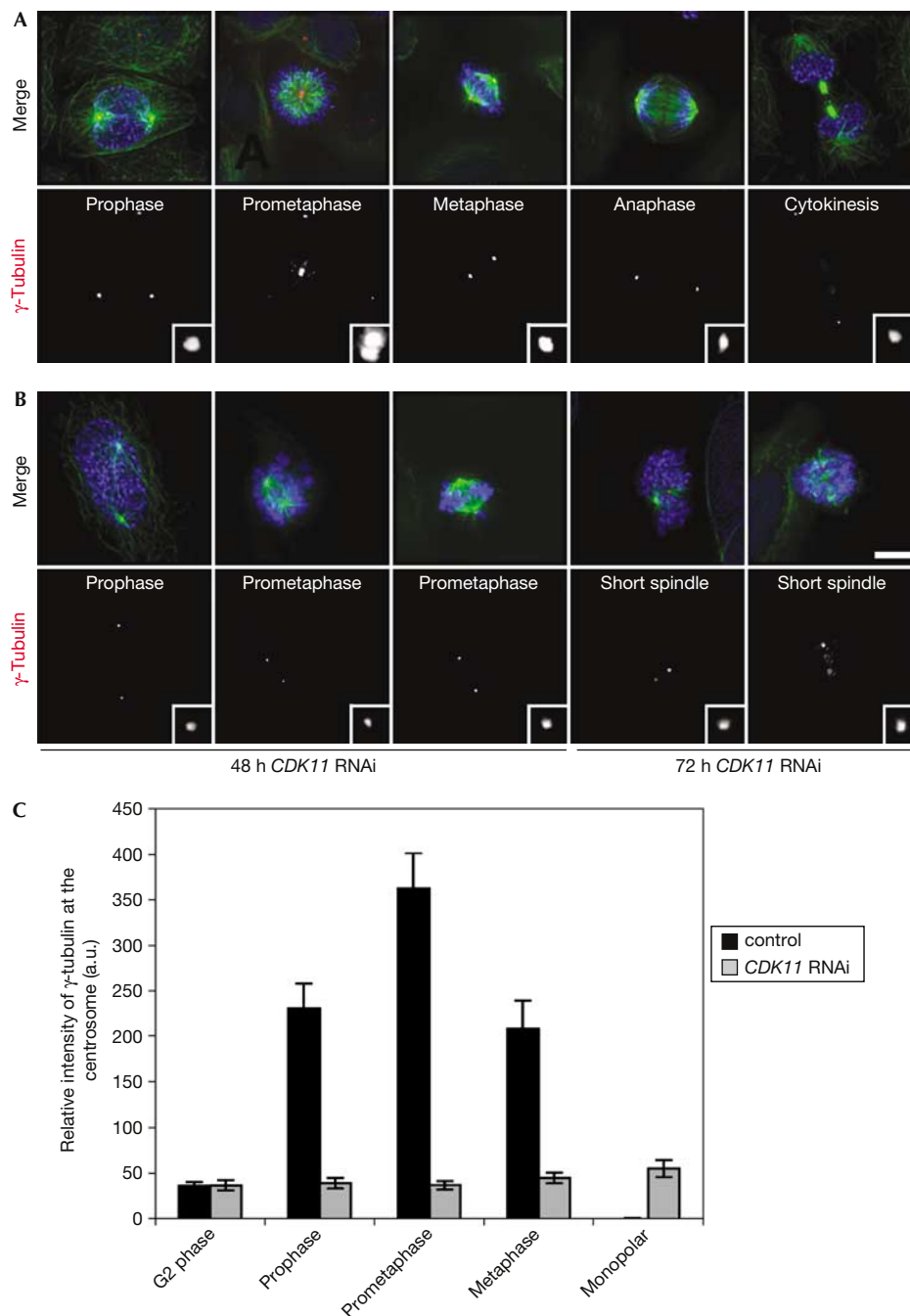


Fig 2 | Cyclin-dependent kinase 11 RNA interference leads to monopolar and short spindles with diminished levels of centrosomal γ -tubulin. Distribution of β -tubulin (green), γ -tubulin (red and lower panels in monochrome) and DNA (blue) throughout mitosis in control (A) or CDK11 (cyclin-dependent kinase 11) RNA interference (RNAi)-treated cells (B). During mitosis, γ -tubulin is recruited to the centrosomes and reaches a maximum during prometaphase (insets). (B) The spindles in CDK11 RNAi cells are short and one or more chromosomes often lag at the spindle poles 48 h after treatment. At 72 h after treatment, most chromosomes show congression defects on spindles that are poorly defined or are monopolar. Note the hypercondensed chromosomes in these cells. Scale bar, 10 μ m. (C) Relative quantification of γ -tubulin immunofluorescence signal intensity at the centrosomes in control (black) and CDK11 RNAi-treated cells (grey). In controls, centrosomal γ -tubulin levels are maximal during prometaphase, but consistently at least 5–7 times higher in mitosis relative to G2 stages. After depletion of CDK11, the centrosomal γ -tubulin signal intensity does not increase beyond G2 levels.

therefore performed microtubule regrowth assays in HeLa cells expressing GFP-tagged α -tubulin (Fig 3B; Meraldi & Nigg, 2001). Following cold-mediated microtubule depolymerization, both

control and RNAi-treated cells were returned to 37 °C for 1, 2 or 3 min and then fixed for fluorescence analysis. The centrosomes in mitotic control cells nucleated robust microtubule asters in the

Table 1|Quantification of mitotic defects after 48 and 72 h *CDK11* RNAi in HeLa cells

Transfection time	Treatment	Mitotic index*	Prophase*	Prometa-phase*	Metaphase*	Anaphase+telophase*	Cytokinesis*	Abnormal metaphase plate*‡	Monopolar+ short spindles*
48 h	Control	8.3 ± 0.5	4.4 ± 2.3	5.7 ± 1.3	16.0 ± 2.2	3.5 ± 1.3	70.1 ± 2.7	0	0
	<i>CDK11</i> RNAi	15.9 ± 0.9	3.5 ± 0.6	15.6 ± 1.8	5.7 ± 2.4	1.9 ± 1.0	45.8 ± 8.0	4.5 ± 2.8	22.5 ± 3.4
72 h	Control	9.9 ± 0.3	4.3 ± 2.4	2.5 ± 1.2	24.8 ± 1.4	2.8 ± 1.9	65.5 ± 3.3	0	0
	<i>CDK11</i> RNAi	32.0 ± 1.8	1.9 ± 0.7	1.1 ± 0.1	3.5 ± 0.8	0.7 ± 0.6	27.9 ± 1.1	4.0 ± 0.6	60.8 ± 1.8

CDK11, cyclin-dependent kinase 11; RNAi, RNA interference.
*Values are a percentage of the total mitosis in each category ± s.d.
‡Metaphase plate with several unaligned chromosomes.

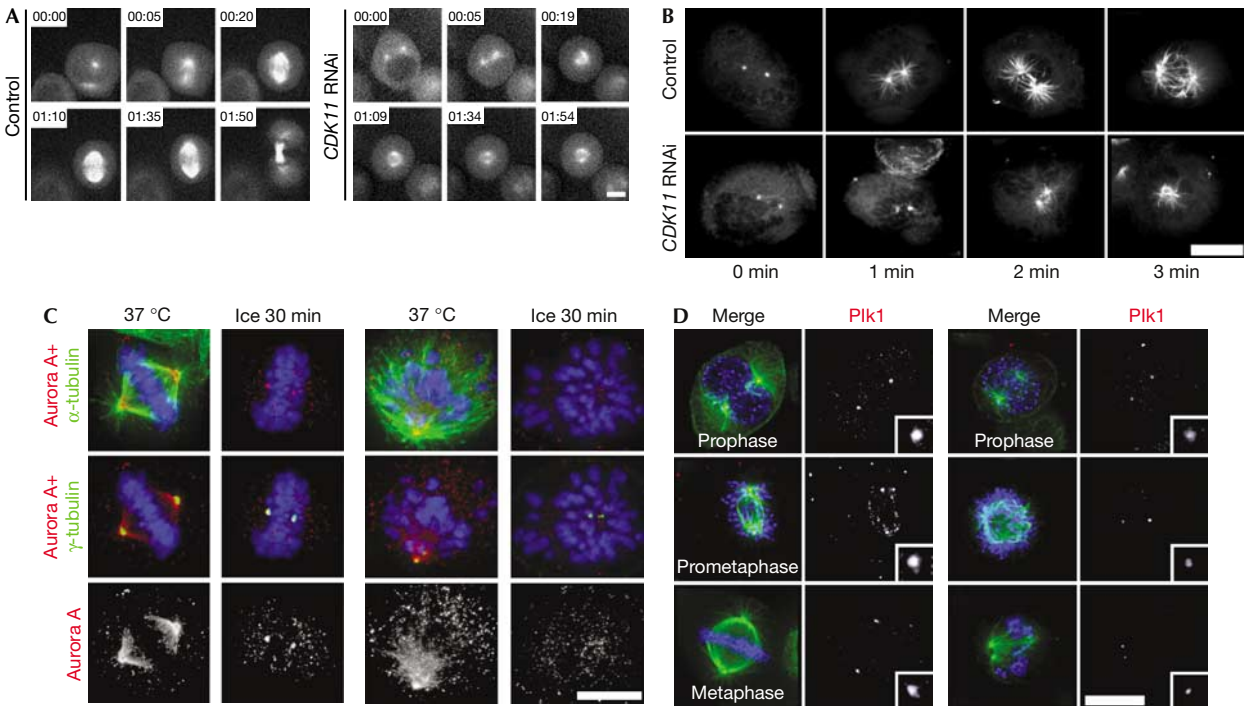


Fig 3 |Cyclin-dependent kinase 11 depletion prevents efficient bipolar spindle formation, microtubule nucleation and centrosomal recruitment of Aurora A and Plk1 proteins. (A) Selected frames from time-lapse recordings of mitosis in a control (left) and a *CDK11* (cyclin-dependent kinase 11) RNA interference (RNAi)-treated cell (right) expressing green fluorescent protein (GFP)-tagged α -tubulin. In control cells, the centrosomes are already partially separated by prophase (00:00) and maintain this separation or move further apart during spindle formation (00:05). By metaphase (00:20–01:10), the spindle reaches a steady-state length before elongating after anaphase onset (01:35) and cytokinesis (01:50; supplementary Movie 1 online). In this *CDK11*-depleted cell, the centrosomes were partially separated during prophase (00:00) but did not undergo any further movement, resulting in monopolar spindle and mitotic arrest (01:54; supplementary Movie 2 online). Scale bar, 5 μ m. (B) *CDK11* RNAi-treated cells have reduced microtubule nucleating potential. A microtubule regrowth assay was performed following cold-induced depolymerization in control (top) and *CDK11*-depleted cells (bottom) expressing GFP-tagged α -tubulin. In control cells, the centrosomes nucleate robust microtubule asters after being returned to 37 °C for 1 and 2 min. Centrosomes in *CDK11* RNAi cells fail to nucleate more than a few short microtubules. After 3 min, a period in which control cells start to form a bipolar spindle, only atrophied asters can be detected in *CDK11* RNAi cells. Scale bar, 10 μ m. (C) Localization of Aurora A in the presence (37 °C) or absence of microtubules (ice) in control (left panels) or *CDK11*-depleted mitoses (right panels). In a control metaphase cell (left), Aurora A (red and lower panel) localizes with the centrosomes (γ -tubulin; green in the middle panels) and spindle microtubules (α -tubulin; green in the top panels). After cold-induced microtubule depolymerization, the kinase still associates with the centrosomes. In the presence of microtubules, *CDK11* knockdown (left panels) does not prevent centrosome or spindle microtubule association of Aurora A. In the absence of microtubules, *CDK11* RNAi cells fail to accumulate Aurora A kinase at their centrosomes. (D) Efficient recruitment of Plk1 to centrosomes and kinetochores requires *CDK11*. In control cells, Plk1 (red in the left-hand panels, insets at right are enlargements of the bottom-most centrosome in each corresponding panel) accumulates at the centrosomes and kinetochores as cells progress into metaphase. In *CDK11* RNAi cells (right panels), the levels of Plk1 at the centrosomes and kinetochores are markedly reduced (compare control and *CDK11* RNAi columns). Scale bar, 10 μ m, insets are a $\times 5$ magnification.

first minute, and by the third minute nascent spindles could be detected (Fig 3B). By contrast, the centrosomes in *CDK11* RNAi cells were difficult to detect and the asters consisted of only a few short microtubules after 1 min (Fig 3B). Prolonging the time did not substantially increase aster density. Thus, in *CDK11* knockdown cells, γ -tubulin is not properly recruited to the centrosome and the microtubule nucleation potential of this structure is diminished.

Aurora A and Plk1 recruitment requires CDK11

The phenotype of *CDK11* RNAi cells was similar to that observed after Aurora A or Plk1 gene disruptions (Giet *et al*, 2002; Sumara *et al*, 2004; Van Vugt *et al*, 2004). We therefore examined whether the localizations of Aurora A and Plk1 protein kinases were altered after *CDK11* RNAi (Fig 3C,D). Western blots (supplementary Fig S2 online) of total cell extracts from asynchronous cultures of control (supplementary Fig S2 online, lane C) and *CDK11*-depleted cells (supplementary Fig S2 online, lane R) indicated that both Aurora A and Plk1 proteins were increased (supplementary Fig S2A online, middle and lower panels). We believe that this reflects an increase in mitotic cells, a stage in which Aurora A and Plk1 levels are maximal (Nigg, 2001; Barr *et al*, 2004).

Immunostaining showed that Aurora A localized to spindles of both control (Fig 3C, left panels) and *CDK11*-depleted cells (Fig 3C, right panels). Therefore, *CDK11* is not needed for the recruitment of Aurora A protein kinase to the spindle. The localization of Aurora A on spindle microtubules makes it difficult to assess its centrosome association. Therefore, microtubules were depolymerized by cold treatment before cell fixation and staining (Fig 3C). Aurora A was consistently detected at the centrosomes of control (Fig 3C, lower panel) but not of *CDK11* RNAi cells.

We next investigated whether Plk1 distribution was disturbed after *CDK11* knockdown (Fig 3D). In control cells, Plk1 (Fig 3D, left panels) associates with the centrosomes from prophase (Fig 3D, upper panels) to metaphase (Fig 3D, lower panels). During prometaphase, we also detected Plk1 on the kinetochores of the congressing chromosomes, although this signal decreased as the chromosomes aligned at the metaphase plate (Fig 3D, compare middle panels with lower panels). Depletion of *CDK11* diminished Plk1 staining at the centrosomes as well as the kinetochores. Quantification of Plk1 centrosomal staining intensity showed a 2–3 factor diminution relative to controls in all mitotic stages examined: prophase, prometaphase and metaphase (supplementary Fig S3 online; Fig 3D, compare upper panels and insets). Monopolar spindles, which remain arrested for prolonged periods of time, had similarly reduced centrosomal Plk1 staining (Fig 3D, middle and lower panels). Therefore, *CDK11* RNAi limits the amount of Plk1 on the centrosome.

GFP-CDK11^{p58} rescues *CDK11* RNAi mitotic phenotype

Immunostaining studies indicated that a pool of CDK11 localized to mitotic centrosomes. To determine whether CDK11^{p58}, CDK11^{p110} or both isoforms are associated with these structures, we generated two human cell lines stably expressing GFP-CDK11^{p58} or GFP-CDK11^{p110} protein kinases (Fig 4A). To prevent the translation of the p58 isoform in GFP-CDK11^{p110} cells, the IRES sequence was mutated. We further introduced five silent mutations in the open reading frame of both GFP transgenes to

give resistance to the siRNA. The endogenous CDK11^{p110} protein kinase was detected in HeLa, GFP-CDK11^{p58} and GFP-CDK11^{p110} cell samples (Fig 4A). In addition, 90- and 140-kDa proteins corresponding to GFP-CDK11^{p58} and GFP-CDK11^{p110}, respectively, were shown using anti-CDK11 (Fig 4A, left panel) and anti-GFP antibodies (Fig 4A, middle panel). Immunoprecipitates against the GFP-CDK11^{p58} and GFP-CDK11^{p110} proteins during interphase (Fig 4B, lanes I) or mitosis (Fig 4B, lanes M) were subjected to western blotting (Fig 4B, upper panel) and *in vitro* histone H1 kinase assays (Fig 4B, middle panel). The kinase activity of GFP-CDK11^{p58} was two times higher during mitosis than during interphase (Fig 4B, lower panel). Conversely, GFP-CDK11^{p110} was four times more active during interphase than during mitosis.

We next studied the distribution of GFP-CDK11^{p58} and GFP-CDK11^{p110}. In contrast to our immunofluorescence study, GFP-CDK11^{p58} (Fig 4D) was diffusely nuclear and cytoplasmic during interphase and was not detectable at the centrosomes (Fig 4D, panel 1). This discrepancy may be due to variations in the fixation procedures used in the experiments. Entry into mitosis was marked by the recruitment of the kinase to the centrosomes (Fig 4D, panel 2). GFP-CDK11^{p58} continued to concentrate on centrosomes during prometaphase and metaphase (Fig 4D, panels 3,4), but by telophase and cytokinesis (Fig 4D, panel 5) this signal was not detectable above the cytoplasmic level seen throughout the cell cycle. By contrast, GFP-CDK11^{p110} (supplementary Fig S4 online) was never observed on centrosomes and was found in mitotic cytoplasm during karyokinesis. Following nuclear reformation at telophase, the p110 isoform concentrated into nuclei where it remained during interphase. These data suggest that the p58 isoform and not the p110 CDK11 isoform has a mitotic function.

To confirm a mitotic role of the p58 isoform, rescue experiments were performed on the GFP-CDK11 cell lines following *CDK11* RNAi. As shown in Fig 4C, the silent mutations engineered into these transgenes conferred resistance to the RNAi, whereas the endogenous proteins were depleted. None of the short or monopolar spindles associated with knockdown of endogenous CDK11 were seen in GFP-CDK11^{p58}-expressing cells (Fig 4E, compare panel 1 with panels 3,4). Further analysis showed that expression of the mutant CDK11^{p58} transgene completely rescued mitotic progression (Table 2). No rescue was observed in GFP-CDK11^{p110} cells after *CDK11* RNAi, and abnormal spindles and mitotic arrest were prevalent (Fig 4E, panel 2; Table 2). These data indicate that CDK11^{p58} but not CDK11^{p110} is a mitotic kinase that localizes to and regulates the maturation of centrosomes for bipolar spindle formation.

DISCUSSION

In this paper, we demonstrate for the first time a role for the human *CDK11* gene in mitosis. We show that the p58 isoform, but not the p110 isoform, is a centrosome-associated mitotic kinase involved in centrosome maturation and bipolar spindle formation. The regulatory partners of CDK11^{p58} are at present unknown. We found no evidence for the association of CDK11^{p58} with the mitotic cyclins A or B or with cyclin L (supplementary Fig S6 online). This latter finding suggests that the kinase activity of p58 is regulated in a manner different from that of the p110 isoform (Xiang *et al*, 1994).

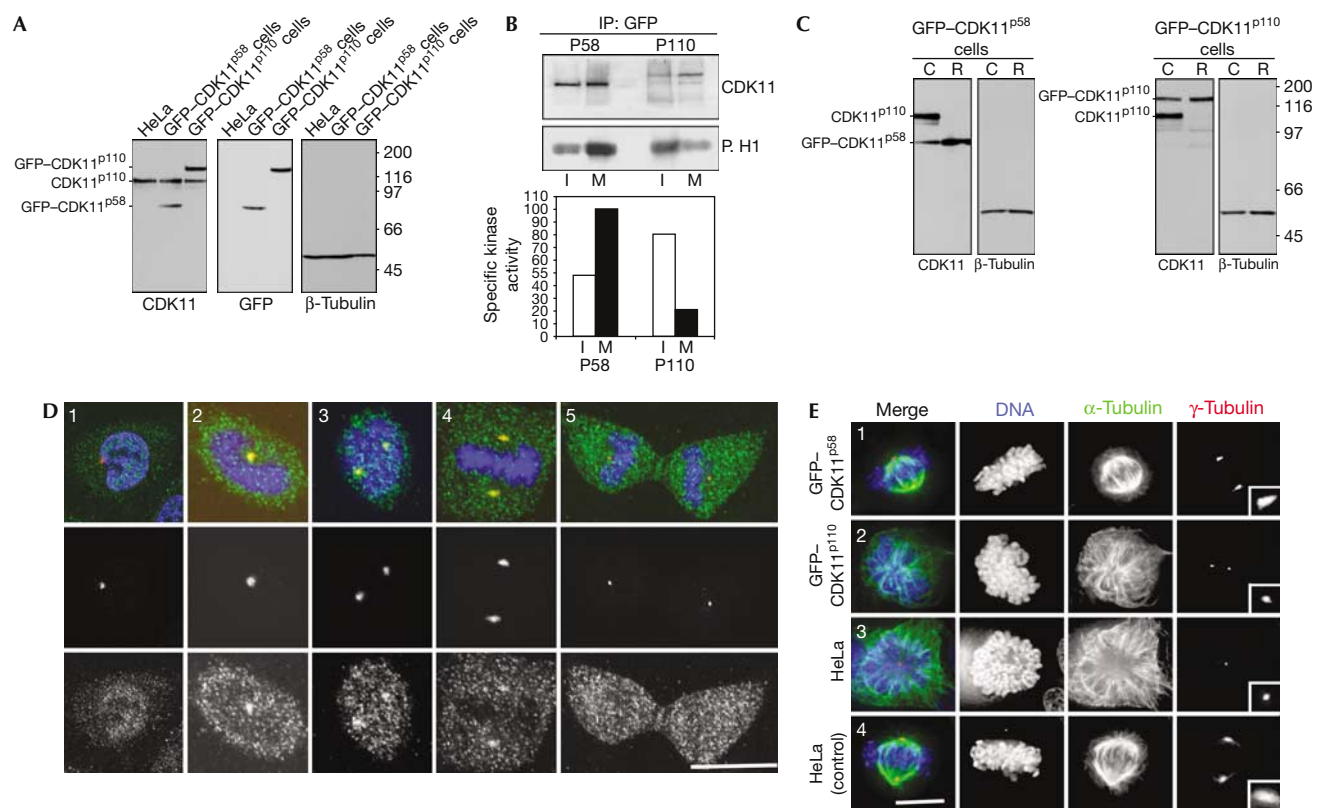


Fig 4 | Green fluorescent protein (GFP)–CDK11^{p58} but not GFP–CDK11^{p110} is a centrosome-associated active mitotic kinase. (A) Western blots of cell extracts from HeLa cells and HeLa transgenic cell lines expressing GFP–CDK11^{p58} and GFP–CDK11^{p110}. β -Tubulin is the loading control. (B) Immunoprecipitation (IP) was performed with GFP–CDK11^{p58} (left) and GFP–CDK11^{p110} (right) cell extracts during interphase (I) or mitosis (M), and the recovered proteins were subjected to a kinase assay. The upper panel shows a CDK11 western blot and the lower panel, the corresponding histone H1 kinase assay. GFP–CDK11^{p58} protein kinase is more active during mitosis than interphase, whereas GFP–CDK11^{p110} is more active during interphase than mitosis. Specific kinase activity determined is shown in the right panel. (C) Western blots of GFP–CDK11^{p58} and GFP–CDK11^{p110} cell lines treated with *CDK11* short interfering RNA (siRNA). The engineering of silent mutations into each of the GFP–CDK variants makes them resistant to *CDK11* RNA interference (RNAi; R), whereas the endogenous proteins are depleted relative to the controls (C). (D) Localization of GFP–CDK11^{p58}. Cells were fixed and stained with an anti-GFP antibody (green and lower panels), anti-pericentrin antibody (red) and co-stained for DNA (blue). GFP–CDK11^{p58} can be detected in the nucleus and cytoplasm during interphase (1). Following mitotic entry (2), GFP–CDK11^{p58} is found in the cytoplasm and at the centrosomes. The kinase remains associated with the centrosome during prometaphase (3) and metaphase (4), but is no longer detectable on centrosomes during cytokinesis (5). Scale bar, 10 μ m. (E) GFP–CDK11^{p58} but not GFP–CDK11^{p110} can rescue the CDK11 RNAi phenotype. The different cell lines were treated with *CDK11* RNAi (1–3) and the distribution of tubulin (green), γ -tubulin (red) and DNA (blue) was examined. GFP–CDK11^{p58} cells depleted of CDK11 (1) were indistinguishable from control cells (4), and formed bipolar spindles. Treatment of GFP–CDK11^{p110} cells with *CDK11* siRNA (2) resulted in the same short or monopolar spindle phenotype seen in HeLa cells (3). Scale bar, 10 μ m.

Table 2 | Quantification of mitotic defects after 48 h *CDK11* RNAi in GFP–CDK11^{p58} and GFP–CDK11^{p110} cells

Cell line	Treatment:	Mitotic index	Prophase*	Prometaphase*	Metaphase*	Anaphase+telophase*	Cytokinesis*	Monopolar+ short spindles*
GFP–CDK1 ^{p58}	Control	10.3 \pm 0.6	1.5 \pm 0.5	5.1 \pm 1.2	13.2 \pm 2.1	1.5 \pm 0.6	78.7 \pm 3.6	0
	<i>CDK11</i> RNAi	11.3 \pm 1.4	1.5 \pm 0.6	4.4 \pm 0.8	13.8 \pm 1.6	1.5 \pm 0.6	78.9 \pm 2.7	0
GFP–CDK ^{p110}	Control	6.9 \pm 0.3	6.2 \pm 2.1	13.8 \pm 0.9	21.1 \pm 3.4	5.9 \pm 1.0	53.0 \pm 1.2	0
	<i>CDK11</i> RNAi	21.4 \pm 3.1	3.3 \pm 0.7	11.4 \pm 2.9	4.4 \pm 0.4	4.9 \pm 1.9	43.5 \pm 3.0	34.7 \pm 2.0

CDK11, cyclin-dependent kinase 11; RNAi, RNA interference.
*Values are a percentage of the total mitosis in each category \pm s.d.

How does CDK11^{p58} control spindle bipolarity? Our time-lapse studies indicated that knockdown of *CDK11* prevented proper centrosome separation. We cannot rule out a role for CDK11^{p58} in recruiting or activating the motor proteins involved in this process. However, we observed that *CDK11* depletion diminished the centrosomal levels of γ -tubulin, Plk1 and Aurora A, proteins with known roles in microtubule nucleation and spindle stabilization (Giet et al, 2002; Sumara et al, 2004; Van Vugt et al, 2004). It is therefore possible that *CDK11* RNAi perturbs centrosome separation not by affecting the motors themselves, but by altering the numbers and/or stability of the microtubule lattices on which they move. Indeed, in the absence of CDK11, centrosomes nucleate far fewer microtubules. Recruitment of the microtubule nucleating protein γ -tubulin to the centrosome requires Plk1 activity, and this family of kinases is in turn regulated by phosphorylation (Qian et al, 1999; Elia et al, 2003). Thus, it is tempting to speculate that Plk1 is a CDK11^{p58} substrate. It will be of interest to test this possibility and to identify other substrates of this new mitotic kinase and understand the molecular mechanisms by which it controls centrosome composition.

METHODS

Cell culture, synchronization and transfection. HeLa cells and HeLa cells stably expressing GFP-tagged α -tubulin, GFP-CDK11^{p58} or GFP-CDK11^{p110} were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Cells were synchronized by thymidine double block. Mitotic cells were collected by mitotic shake off after incubating in 1 μ g/ml nocodazole for 16 h. Transfection of plasmids was performed using calcium phosphate.

Microtubule regrowth assays. GFP-tagged α -tubulin-expressing cells were transfected with *CDK11* or control siRNA oligonucleotides. After 72 h, the microtubules were depolymerized by placing the cells on ice for 30 min. These cells were then incubated with warm (37 °C) medium for 1, 2 or 3 min and fixed with -20 °C methanol and processed for immunofluorescence microscopy (see the supplementary information online).

Oligonucleotides, siRNAs, antibody production and manipulation. See the supplementary information online.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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